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GRAPEFRUIT PHENOLICS—II.

PRINCIPAL AGLYCONES OF ENDOCARP AND PEEL AND THEIR POSSIBLE BIOSYNTHETIC RELATIONSHIP

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Abstract—Umbelliferone, esculetin, scopoletin, bergaptol, *p*-coumaric acid, caffeic acid, ferulic acid, naringenin, isosakuranetin, eriodictyol, hesperetin, apigenin, dihydrokaempferol, kaempferol, quercetin, and isorhamnetin were isolated from extracts of mature grapefruit endocarp (*Citrus paradisi* Macf.) after enzymic hydrolysis of the glycosides and esters. Peel contained the same compounds as endocarp but in different relative amounts. This appears to be the most complete sequence of stable postulated biosynthetic intermediate aglycones leading from *p*-coumaric acid to coumarin, furocoumarin, flavanone, flavone, and flavonol end-products yet found to co-occur in a single plant tissue.

INTRODUCTION

KNOWLEDGE of the phenolic constituents of grapefruit (*Citrus paradisi* Macf.) is very limited. This is especially true of the edible portion of the fruit, the endocarp. It is known that the flavanone neohesperidosides and to a lesser extent the triterpenoid limonin are primarily responsible for the bitter taste of grapefruit. Excessive bitterness when it occurs is recognized as an important factor in limiting the acceptance of grapefruit. Therefore, a comprehensive study of the phenolic constituents of grapefruit and their biosynthetic relationship to the bitter flavanones has been undertaken to help find a solution to this problem.

The presence of the bitter flavanone glycoside naringin in grapefruit has been known for many years;[1] however, it was not until the work of Horowitz and Gentili in 1963[2] that the complete structure of naringin was determined. These workers[3] also reported the presence of phlorin and the bitter flavanone glycoside poncirin in grapefruit peel, and Rowell and Beisel[4] found rhoifolin in peel and rag. Stanley, *et al.*[1a, 5] found three highly methylated flavones, bergaptol, and a variety of alkylated coumarins and psoralens[6] in grapefruit peel oil. Fisher and Nordby[7, 8] identified limettin and additional alkylated coumarins and psoralens of peel

* A laboratory of the Western Utilization and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

[1] For reviews see:

[a] R. M. HOROWITZ, In *The Orange, Its Biochemistry and Physiology* (Edited by W. B. SINCLAIR) p. 334. University of California Press, Berkeley (1961).

[b] J. W. KESTERSON and R. HENDRICKSON, *Florida Univ. Agr. Expt Sta. Bull.* No. 511A (1957).

[2] R. M. HOROWITZ and B. GENTILI, *Tetrahedron* **19**, 773 (1963).

[3] R. M. HOROWITZ and B. GENTILI, *Arch. Biochem. Biophys.* **92**, 191 (1961).

[4] K. M. ROWELL and C. G. BEISEL, *J. Food Sci.* **28**, 195 (1963).

[5] W. L. STANLEY, S. H. VANNIER, L. PETRICEKS and R. E. LUNDIN, In preparation.

[6] W. L. STANLEY, In *Aspects of Plant Phenolic Chemistry, Proc. 3rd Ann. Symp.* p. 79. Plant Phenolics Group of North America, Toronto (1963).

[7] J. F. FISHER and H. E. NORDBY, *J. Food Sci.* **30**, 869 (1965).

[8] J. F. FISHER and H. E. NORDBY, *Tetrahedron* **22**, 1489 (1966).

oil. Dunlap and Wender[9] reported the presence of neohesperidin and kaempferol in a commercial grapefruit flavonoid preparation.

Until very recently the only phenols isolated from grapefruit endocarp were naringin and rhoifolin. However, in 1965 Wheaton and Stewart[10] reported the presence of feruloylputrescine in grapefruit juice; Mizelle *et al.*[11] identified naringenin-β-rutinoside, isosakuranetin-β-rutinoside, poncirin, hesperidin, and neohesperidin in Ruby Red grapefruit segments; and Maier and Dreyer[12] reported the isolation of bergaptol from grapefruit juice. Nevertheless, chromatographic examination of grapefruit endocarp extracts shows that many other phenols remain to be identified.

In the first paper of this series we reported the isolation and identification of dihydrokaempferol from grapefruit.[13] In the present paper we report the isolation and identification of most of the principal phenolic aglycones (primarily coumarins, cinnamic acids, and flavonoids) of the endocarp and peel of mature Marsh grapefruit and discuss the biosynthetic interrelationship of these phenols.

TABLE 1. CRITERIA USED IN IDENTIFYING INDIVIDUALLY ISOLATED PHENOLIC COMPOUNDS*

Compound	TLC‖			Paper electrophoresis	U.V. spectra and shifts	M.P.§ and i.r. spectra
	on MCC	on Silica gel G	Color‡ tests			
Bergaptol	x	x	UV, BZ	x	x	x
Umbelliferone	x		UV, BZ	x		
Esculetin†	x		UV, SN			
Scopoletin	x		UV, BZ		x	x
p-Coumaric acid	x		UV, BZ	x		
Caffeic acid	x		UV, BZ, SN	x	x	x
Ferulic acid	x		UV, BZ	x	x	x
Naringenin	x		UV, BZ, BR		x	x
Isosakuranetin	x	x	UV, BZ, BR		x	
Eriodictyol	x		UV, BZ, BR, SN		x	
Hesperetin	x		UV, BZ, BR		x	
Dihydrokaempferol	x		UV, BZ, BR	x	x	
Apigenin	x	x	UV, BZ, BR		x	
Kaempferol	x	x	UV, BZ, BR		x	
Quercetin	x		UV, BZ, BR, SN		x	
Isorhamnetin	x		UV, BZ, BR		x	
Phloroglucinol	x	x	UV, BZ			

* "x's" and symbols used for color tests mean the grapefruit compound was identical to authentic compound in the property or test listed.

† Identification tentative.

‡ UV=appearance under ultra-violet light before and after fuming with ammonia vapor; BZ=color after spraying with bis-diazotized benzidene; BR=color after spraying with sodium borohydride and fuming with hydrogen chloride; SN=color after spraying with ammoniacal silver nitrate.

§ Melting point and mixed melting point.

‖ Compounds were compared with authentic specimens in at least three solvents on both MCC (microcrystalline cellulose) and silica gel G.

[9] W. J. DUNLAP and S. H. WENDER, *Anal. Biochem.* **4**, 110 (1962).
[10] T. A. WHEATON and I. STEWART, *Nature* **206**, 620 (1965).
[11] J. W. MIZELLE, W. J. DUNLAP, R. E. HAGEN, S. H. WENDER, B. J. LIME, R. F. ALBACH and F. P. GRIFFITHS, *Anal. Biochem.* **12**, 316 (1965).
[12] V. P. MAIER and D. L. DREYER, *J. Food Sci.* **30**, 874 (1965).
[13] V. P. MAIER and D. M. METZLER, *Phytochem.* **6**, 763 (1967).

RESULTS

Seventeen phenols (Table 1 and Fig. 1) were isolated from extracts of grapefruit endocarp following enzymic hydrolysis of glycosides and esters. (A number of minor phenols remain to be identified.) Advantage was taken of the high resolving power and speed of preparative thin-layer chromatography (TLC) to isolate the compounds in pure form. Their identities were established by direct comparison with authentic samples by means of TLC, u.v. spectra and spectral shifts, paper electrophoresis, and color tests. Several compounds were also isolated in crystalline form by column chromatographic techniques. Two-dimensional TLC comparison of endocarp extracts before and after hydrolysis showed that all of these phenols, except bergaptol, occurred in the fruit primarily in bound forms (glycosides and esters) that

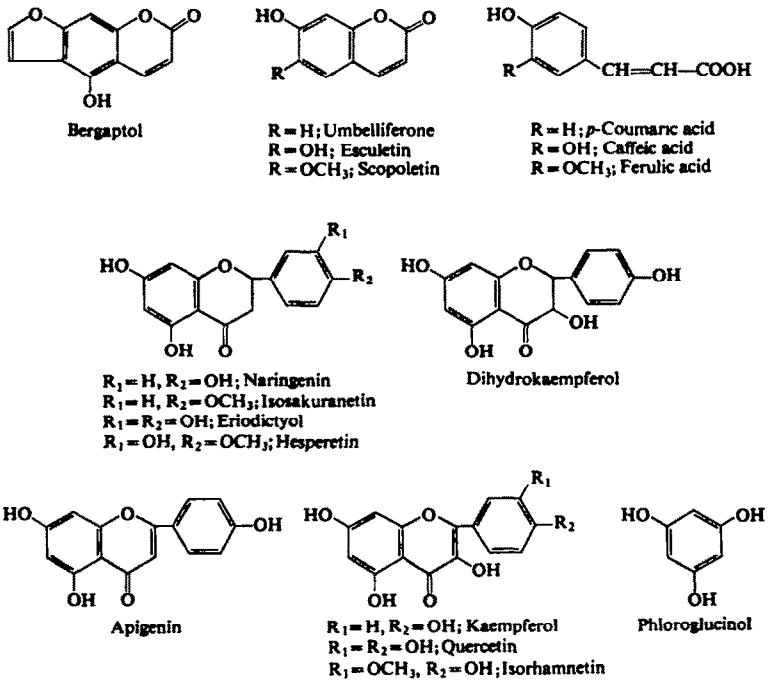

FIG. 1. PRINCIPAL PHENOLIC AGLYCONES OF GRAPEFRUIT.

are enzymically hydrolyzable. A significant proportion of the bergaptol occurred in both the free and bound forms. Very small amounts of free umbelliferone and scopoletin were found but they may be artifacts caused by a minor amount of hydrolysis during sample preparation. It is possible that a portion, or possibly all, of the bound coumarins occur naturally as glycosides[14] or esters of their corresponding *cis*-orthohydroxycinnamic acids. These acids would undergo spontaneous lactonization to coumarins after hydrolysis.

Treatment of endocarp extracts with β-glucosidase followed by two-dimensional TLC on microcrystalline cellulose gave significant amounts of phloroglucinol, caffeic acid, ferulic acid, scopoletin, umbelliferone and a trace of naringenin. Thus, a significant portion of the phloroglucinol, caffeic acid, ferulic acid, scopoletin and umbelliferone appear to occur as glucosides. A trace of naringenin also appears to occur as a glucoside.

[14] S. A. BROWN, *Phytochem.* 2, 137 (1963).

Grapefruit peel contained the same seventeen phenols as the endocarp but in different relative amounts, Table 2. As with the endocarp, the phenols were present in bound forms (with one exception) and were released by enzyme hydrolysis. Bergaptol was present only in the free form in peel.

The endocarp and peel chloroform-soluble fractions contained significant amounts of bergaptol and traces of scopoletin and umbelliferone, but none of the other phenols listed in Table 1, when assayed by two-dimensional TLC on microcrystalline cellulose. However, both of these fractions showed numerous fluorescent spots (38 in endocarp and 30 in peel)

TABLE 2. RELATIVE COMPOSITION OF THE ENTIRE ENDOCARP AND PEEL OF A TYPICAL MATURE GRAPEFRUIT*

Occurring as glycosides and esters	Endocarp†	Peel†
Bergaptol	2	—
Umbelliferone	1	5
Esculetin	T	T
Scopoletin	4	1
p-Coumaric acid	3	2
Caffeic acid	4	2
Ferulic acid	5	5
Naringenin	10	50
Isosakuranetin	2	5
Eriodictyol	1	1
Hesperetin	2	1
Apigenin	3	7
Dihydrokaempferol	2	2
Kaempferol	2	T
Quercetin	2	1
Isorhamnetin	2	T
Phloroglucinol	2	14
Occurring as the free phenol		
Bergaptol	3	2

* Numbers represent relative sizes and intensities of spots on TLC, T = trace, and "—" = absent (see text).

† Values can be converted from a "per fruit basis" to a "per unit weight of wet tissue basis" by multiplying endocarp values by 1 and peel values by 2·36.

when assayed by TLC on silica gel G, a better system for separating relatively non-polar compounds. It is likely that many of the fluorescent spots are alkylated coumarins and psoralens and highly-methylated flavonoids similar to those previously reported in grapefruit peel oil.[5–8]

DISCUSSION

Although grapefruit contain large amounts of flavanones the fruit also possess a wide array of other phenolic constituents which have previously received little attention. Among the compounds identified, *p*-coumaric acid, caffeic acid, eriodictyol, quercetin, and isorhamnetin

are reported here for the first time in grapefruit. We recently reported the presence of bergaptol and dihydrokaempferol in various grapefruit tissues.[12, 13]

The compounds identified in grapefruit, when arranged according to the most commonly accepted postulated biosynthetic pathways,[15a, 15b] reveal the most complete sequence of stable aglycone intermediates leading from *p*-coumaric acid to coumarin, furocoumarin, flavanone, flavone, and flavonol end-products yet found to co-occur in a single plant tissue

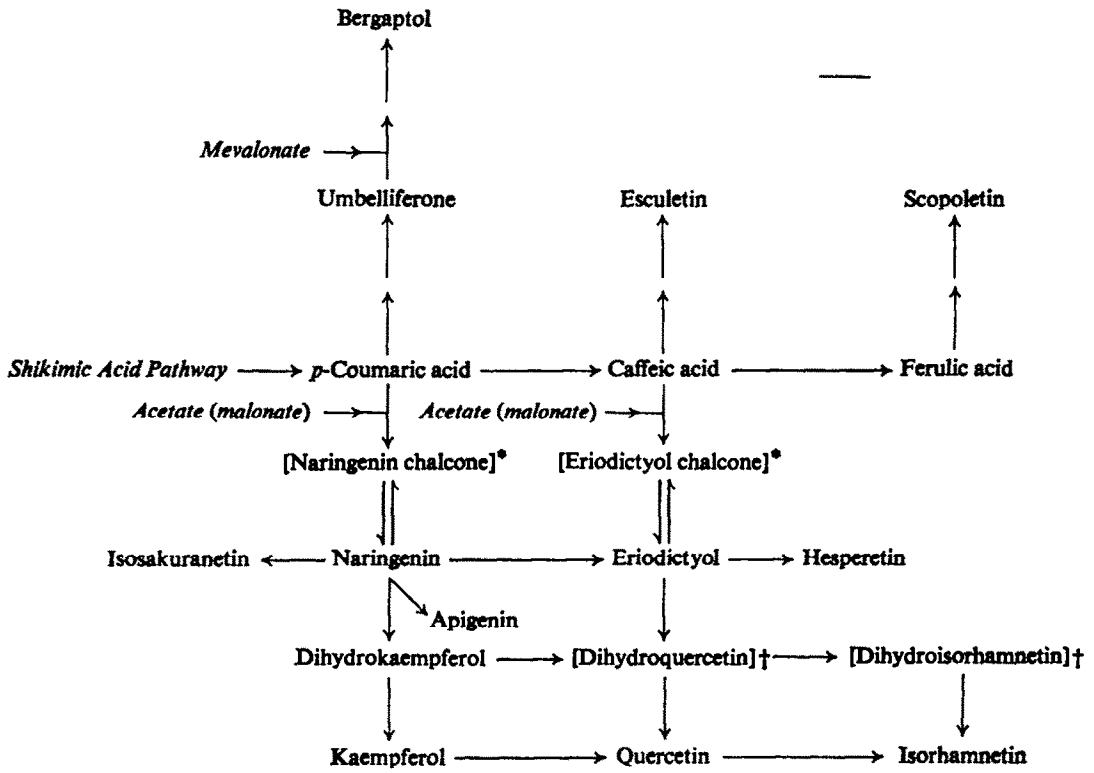

FIG. 2. POSSIBLE INTERRELATIONSHIP OF GRAPEFRUIT PHENOLIC COMPOUNDS BASED ON CURRENTLY POSTULATED PATHWAYS FOR BIOSYNTHESIS OF FUROCOUMARINS, COUMARINS, CINNAMIC ACIDS, AND FLAVONOIDS.

* Compounds not found. They would not be expected to be isolated because the equilibria favor the flavanone forms.

† Compounds not found but whose presence is suspected on the basis of preliminary evidence.

(Fig. 2). For those compounds having *p*-hydroxy ring substituents (B-ring of flavonoids) each postulated stable aglycone intermediate leading to umbelliferone, apigenin and kaempferol is present. Since the equilibrium between 2′,4′,6′-trihydroxychalcones and their respective

[15] For reviews see:

[a] H. GRISEBACH, In *Chemistry and Biochemistry of Plant Pigments* (Edited by T. W. GOODWIN), p. 279. Academic Press, New York (1965).

[b] J. D. BU'LOCK, *The Biosynthesis of Natural Products, An Introduction to Secondary Metabolism.* McGraw-Hill, New York (1965).

flavanones lies almost entirely on the side of the flavanones, isolation of 2',4',6',4-tetrahydroxychalcone would not be expected.[16] Also, isolation of *cis*-2,4-dihydroxycinnamic acid, released by hydrolysis of its glucosides (which are believed to be intermediates in umbelliferone biosynthesis),[17–19] would not be expected because it would spontaneously lactonize to umbelliferone. Bergaptol can be envisioned as arising by a pathway similar to that proposed by Floss and Mothes[20] for the biosynthesis of bergapten and isobergapten in *Pimpinella magma*. These furocoumarins are thought to be formed from cinnamic acid via *p*-coumaric acid and umbelliferone, with the two extra-carbons of the furan ring originating from mevalonic acid. The only stable intermediate of this pathway not yet found in grapefruit is 5,7-dihydroxycoumarin. However, the occurrence of 5,7-dimethoxycoumarin (limettin) in grapefruit[7] suggests that 5,7-dihydroxycoumarin is also formed. The central importance of *p*-coumaric acid is apparent from the map (Fig. 2); all compounds shown can be derived from it.

The presence of isosakuranetin and hesperetin in the absence of cinnamic acids, coumarins, and other flavonoids with the same substitution and methylation patterns suggests that these flavanones are formed in grapefruit via methylation of naringenin and eriodictyol, respectively. This, in turn, suggests the existence of a para-*O*-methyltransferase enyzme specific for flavanones. The presence of ferulic acid and isorhamnetin indicates the presence of meta-*O*-methyltransferase enzymes such as have been found in other plant tissues.[21] Barz, Patschke and Grisebach[22] have shown that dihydrokaempferol, but not kaempferol, is an efficient precursor of quercetin in buckwheat seedlings. Whether kaempferol or dihydrokaempferol (presumably via dihydroquercetin) is a better precursor of quercetin in grapefruit remains to be determined. The presence of dihydroquercetin and dihydroisorhamnetin in grapefruit is uncertain at the moment. We have isolated small amounts of materials that have properties resembling these compounds, but the amounts were not sufficient to permit positive identification. If dihydroquercetin and dihydroisorhamnetin are indeed present one can envision a "metabolic grid" along the lines proposed by Bu'Lock[15b] in which alternative pathways of varying activities lead to dihydroquercetin, quercetin and isorhamnetin. The relatively small amount of esculetin present compared with umbelliferone and scopoletin suggests considerable differences in the amounts or activities of the enzymes that synthesize the coumarins from their respective cinnamic acid precursors. A "metabolic grid" interrelationship can also be envisioned among the cinnamic acids and coumarins, although most (but not all) evidence gathered with other plants favors coumarin formation from the respective cinnamic acids rather than by way of hydroxylation and methylation of umbelliferone or other coumarins.[17–19,23] Phloroglucinol is thought to be synthesized in plants by condensation of acetate units; however, it has been suggested by Horowitz and Gentili[3] that there may be a biosynthetic relationship between inositol and phloroglucinol.

Other pathways than those of Fig. 2 have been proposed for flavonoid biosynthesis.

[16] T. R. SESHADRI, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. GEISSMAN), p. 159. Pergamon Press, Oxford (1962).
[17] For reviews see:
A. C. NEISH, In *Biochemistry of Phenolic Compounds* (Edited by J. B. HARBORNE) p. 295. Academic Press, New York (1964); and G. N. H. TOWERS, *ibid.* p. 249.
[18] D. J. AUSTIN and M. B. MEYERS, *Phytochem.* **4**, 255 (1965).
[19] S. A. BROWN, G. H. N. TOWERS and D. CHEN, *Phytochem.* **3**, 469 (1964).
[20] H. G. FLOSS and U. MOTHES, *Phytochem.* **5**, 161 (1966).
[21] B. J. FINKLE and R. F. NELSON, *Biochim. Biophys. Acta* **78**, 747 (1963).
[22] W. BARZ, L. PATSCHKE and H. GRISEBACH, *Chem. Commun.* 400 (1965).
[23] H. KINDL and G. BILLEK, *Monatsh. Chem.* **95**, 1044 (1964).

Roux,[24] for instance, proposed that flavan 3,4-diols are formed first and subsequently converted into the other flavonoids by a combination of reduction and dehydrogenation reactions. Present knowledge of grapefruit phenolics does not support this propsed pathway since no flavan 3,4-diols have yet been reported. Haslam[25] has suggested the possibility that diverse pathways lead to different flavonoids. He speculates that flavones are formed by the pathway given in Fig. 2 and that dihydroflavonols and flavonols are formed by another pathway in which the original C_{15} compound arises from *p*-hydroxyphenylpyruvic acid. On the basis of the phenolic constituents found in grapefruit this diverse pathways scheme cannot be excluded as a possibility.

At present it is not known whether the phenolic constituents of citrus fruits are synthesized in place or are translocated into the fruit from the leaves. The wide array and apparent interrelation of the grapefruit phenolics reported here suggests that the fruit tissue does have the ability to synthesize or interconvert these compounds, at least starting from the early C_9 or C_{15} stages. The reported isolation of a flavanone synthease enzyme from the peel of several citrus fruits by Shimokoriyama[26] also supports this view.

The main difference in composition between grapefruit peel and endocarp lies in the relative amounts of the individual compounds rather than the nature of the compounds. Based on the total amounts present in the peel and endocarp of a typical fruit the following generalizations can be made: (a) peel contains much larger amounts of phloroglucinol, umbelliferone, apigenin, and total flavanones than does endocarp; (b) endocarp contains much larger amounts of scopoletin and flavonol compounds than does peel; (c) peel and endocarp contain similar amounts of total C_6-C_3 compounds (cinnamic acids plus coumarins); (d) peel contains no bound bergaptol that is enzymically hydrolyzable; and (e) total phenolic material of the endocarp is more uniformly distributed among the individual compounds than it is in peel. On the basis of the relative amounts of compounds per unit weight of peel and endocarp (i.e. concentration of compounds in the wet tissue) the peel exceeds the endocarp in all compounds except scopoletin, bound bergaptol,* kaempferol, and isorhamnetin (Table 2, footnote 1).

All of the bound forms reported in Table 1 are present in the fruit as glycosides or esters (based on the method of hydrolysis). Evidence was also obtained by β-glucosidase hydrolysis that naringenin, phloroglucinol, ferulic acid, caffeic acid, scopoletin, and umbelliferone occur in part as glucosides. Horowitz and Gentili[3] have previously isolated phloroglucinol-β-D-glucoside from grapefruit. By analogy with the reported occurrence of 7-neohesperidosides and 7-rutinosides of the other flavanones, eriodictyol would also be expected to occur in both of these glycosidic forms. Flavanone 7-neohesperidosides are important because they are intensely bitter compounds.[2,3]

Accumulation of large amounts of naringenin glycosides in grapefruit tissues[27] is of considerable interest in light of the position occupied by naringenin in the postulated scheme (Fig. 2). Since pathways for the further metabolism of naringenin appear to be present, accumulation of large amounts of naringin and other flavanone neohesperidosides and rutinosides may be due to the fact that enzymes responsible for further oxidation of naringenin to apigenin and dihydrokaempferol are specific for a form of naringenin that only exists at very

* The bergaptol of endocarp is unique in that it is the only phenolic constituent identified here that is present in significant amounts in both the free and bound forms.

[24] D. G. Roux and E. Paulus, *Biochem. J.* **82**, 324 (1962).
[25] E. Haslam, *Chemistry of Vegetable Tannins*, p. 160. Academic Press, New York (1966).
[26] M. Shimokoriyama, *J. Am. Chem. Soc.* **79**, 4199 (1957).
[27] J. W. Kesterson and R. Hendrickson, *Florida Univ. Agr. Expt Sta. Bull.* No. 511A, Gainsville (1957).

low concentrations. This form may be free naringenin or a naringenin glucoside (we have evidence for the presence of small amounts of the latter) or one of the diastereomeric forms of either of these compounds. Patschke, Barz and Grisebach[28] have shown that the incorporation rate of (−) 5,7,4′-trihydroxyflavanone-5-glucoside-[2-^{14}C] into quercetin in buckwheat seedlings was 16 times higher than that of the (+) enantiomer. Thus, the enzymes leading from the precursor to naringenin 7-neohesperidoside and -rutinoside appear to be much more plentiful than those leading to apigenin and dihydrokaempferol and/or their glycosides.

EXPERIMENTAL*

Mature grapefruit (*Citrus paradisi*, Macf. var. Marsh) were harvested from several trees in a commercial grove located in the desert region of California. The fresh fruit was separated into peel (albedo and flavedo) and endocarp (the edible portion which contains the juice). The seeds were discarded. Each tissue fraction was blended in methanol, heated to inactivate enzymes and repeatedly extracted with methanol until free of flavonoids. Methanol was removed from the extract by vacuum evaporation at 40° leaving an aqueous residue. Glycosides, esters and free phenols were removed from an aliquot of the aqueous residue by repeated liquid: liquid extraction with ethyl acetate containing 10% (v/v) methanol. Another aliquot of the aqueous residue was separated into a $CHCl_3$-soluble fraction and a water-soluble fraction. Removal of $CHCl_3$-soluble constituents before hydrolysis of the water-soluble fraction simplified subsequent TLC of the hydrolyzed phenols. After enzyme hydrolysis phenols were extracted from the water-soluble fraction by repeated liquid: liquid extraction with ethyl acetate containing 10% (v/v) methanol. Anthocyanase (Rohm and Haas) was used for hydrolysis of glycosides and esters, and β-glucosidase (Nutritional Biochemicals Corp.) was used for selective hydrolysis of glucosides.[13]

Both column and preparative thin-layer chromatography (TLC) were used to isolate the phenolic moieties of the anthocyanase hydrolyzed fraction of the endocarp. Separation of a number of the phenols was achieved on a partitioning column in which the support phase was silicic acid of 50% (w/w) water content. A linear gradient of glacial acetic acid in benzene (0–50% v/v) gave the following compounds in the order in which they were eluted: isosakuranetin, scopoletin, bergaptol, ferulic acid, umbelliferone, *p*-coumaric acid, naringenin, and caffeic acid. Other phenols were isolated from the hydrolyzed fraction by preparative TLC using 20 × 20 cm plates coated with a 1 mm layer of microcrystalline cellulose (MCC) (Brinkmann Instruments, Inc.). The extracts was streaked 2 cm from one edge of the plate, the plate was developed with solvent (ascending), the various bands scraped off, eluted with methanol, and restreaked separately on another plate.[13] The nature of the developing solvents and their order of use depended on the compound being isolated. The solvents used were: benzene-acetic acid-water, 6:7:3[29] (BzAW-6) and 125:72:3[30] (BzAW-125); 10, 30 and 50% acetic acid; and water.

Aglycones isolated by column and preparative thin-layer chromatography were directly compared with authentic compounds by one-dimensional TLC on MCC using in each case at least three of the solvents mentioned above. Diagnostic spray reagents used were bis-diazotized benzidene,[31] ammoniacal $AgNO_3$[32] and borohydride-HCl.[33] Authentic samples of all compounds listed in Table 1 were available for comparison.

The relative amounts of the individual aglycones in the endocarp and peel of a typical fruit were estimated by spotting the extracts in amounts such that the weight ratio of endocarp to peel extractives applied to separate chromatograms was the same as the weight ratio of endocarp to peel of an average size fruit (i.e. 2·36:1). Aglycones were assayed by two-dimensional TLC (20 × 20 cm plates) on a 0·25 mm layer of MCC using ascending development with BzAW-125 and after thorough drying 10% HOAc or water. Microcrystalline cellulose gives excellent separations, very tight spots, and is greatly superior to paper chromatography or TLC on powdered cellulose. Each spot was identified by its R_f value, its appearance under u.v. and visible light before and after exposure to ammonia vapor, and by its color when sprayed with chromogenic reagents. Estimation of thr relative amounts of the individual aglycones was made by relating the intensity and size of spot to that of naeingenin. Since all compounds do not have the same molar color intensities this method does not give results in absolute amounts; however, it does allow relative comparison of different compounds and direct comparison between identical compounds of endocarp and peel. The spots were assessed mainly under long

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[28] L. PATSCHKE, W. BARZ and H. GRISEBACH, *Z. Naturforschg.* **21b**, 201 (1966).
[29] L. A. GRIFFITHS, *Nature* **180**, 1373 (1957).
[30] E. WONG and A. O. TAYLOR, *J. Chromatog.* **9**, 449 (1962).
[31] D. G. ROUX and A. E. MAIHS, *J. Chromatog.* **4**, 65 (1960).
[32] S. M. PARTRIDGE and R. G. WESTALL, *Biochem. J.* **42**, 238 (1948).
[33] R. M. HOROWITZ, *J. Org. Chem.* **22**, 1733 (1957).

wavelength u.v. light although viewing under u.v. light after exposure to ammonia vapor and after spraying with bis-diazotized benzidene also aided evaluation. In scoring, (Table 2) the naringenin spot of the peel extract chromatogram was given a value of 50, and the other spots were given an appropriate score by direct comparison. Spots definitely present but in amounts too small to give a numerical value are recorded as *T* (trace). The absence of a spot is represented by "—".

Chloroform soluble compounds were separated by two-dimensional TLC (20 × 20 cm plates) on a 0·25 mm layer of silica gel G using ascending development with benzene-ethanol-water-acetic acid (200:47:15:1)[34] and then toluene:ethyl acetate:formic acid (5:4:1). Spots were observed under u.v. light.

U.V. spectra were determined with a Cary 14 spectrophotometer in ethanol alone, and with added sodium ethoxide, fused sodium acetate, boric acid-sodium acetate, and $AlCl_3$.[35] I.r. spectra were determined in Nujol or KBr with a Perkin Elmer Infracord spectrophotometer. High voltage paper electrophoresis was carried out on Whatman No. 1 filter paper with 0·05M borate buffer (pH 9) and 110 V/cm for 75 min. In all cases the authentic compounds were compared directly with the isolated individual grapefruit compounds as listed in Table 1.

Acknowledgements—We are indebted to Mr. J. C. Pew, Forest Products Laboratory, Madison, Wisconsin for the sample of dihydrokaempferol, to Dr. R. M. Horowitz and Mr. B. Gentili of this laboratory for their generosity in supplying the other phenolic compounds mentioned herein, and to the Schell Ranch, Indio, California for the grapefruit.

[34] G. W. Hay, B. A. Lewis and F. Smith, *J. Chromatog.* **11**, 479 (1963).
[35] L. Jurd, In *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman), p. 107. Pergamon Press, Oxford (1962).